Biochemical and Biophysical Research Communications 441 (2013) 77–82

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Acceleration of adipogenic differentiation via acetylation of malate dehydrogenase 2

Eun Young Kim [a], Baek Soo Han [a,b], Won Kon Kim [a], Sang Chul Lee [a,*], Kwang-Hee Bae [a,b,*]

[a] Research Center for Integrated Cellulomics, KRIBB, Daejeon 305-806, South Korea
[b] Functional Genomics Program, University of Science and Technology (UST), Daejeon 305-806, South Korea

ARTICLE INFO

Article history:
Received 2 October 2013
Available online 14 October 2013

Keywords:
Acetylation
Adipogenesis
MDH2
NADPH
PTMs

ABSTRACT

Previously, we identified proteins showing a differential acetylation pattern during adipogenic differentiation. Here, we examined the role of malate dehydrogenase 2 (MDH2) acetylation in the adipogenesis of 3T3-L1 preadipocytes. The acetylation level of MDH2 showed a dramatic increase during adipogenesis. The overexpression of wild-type MDH2 induced the significant acceleration of adipogenic differentiation. On the other hand, the acetylation-block mutant MDH2 showed significantly reduced adipogenic differentiation compared to the wild type. MDH2 acetylation enhances its enzymatic activity and consequently intracellular NADPH level. These results suggest that the acetylation of MDH2 was affected by the cellular energy state and subsequently regulated adipogenic differentiation.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Post-translational modifications (PTMs) of proteins play crucial roles in cell signaling, protein–protein interaction, protein stability and enzymatic activity. Among a number of PTMs, acetylation on lysine resides of non-histone proteins has emerged as a key PTM for controlling protein functions in both bacteria and mammalian cells [1,2]. Lysine acetylation is catalyzed by lysine acetyltransferases (KATs), and the reverse reaction occurs due to lysine deacetylases (KDACs). Recently, proteomic studies have revealed more than 2000 proteins that are modified by acetylation. However, only a small fraction of these proteins has been investigated to determine how acetylation influences their functions [3–6].

Adipocytes have crucial roles in energy homeostasis, and they store excess energy as triglycerol. Adipogenesis is a delicately controlled cellular differentiation process in which preadipocytes are transformed into mature adipocyte cells [7]. Obesity is a growing health problem worldwide, associated with increased risk of chronic diseases. Obesity is caused by both an increased adipocyte size (hypertrophy) and increased adipocyte numbers (hyperplasia). Therefore, deeper understanding of the molecular basis of adipogenesis is essential to overcome the ill effects of obesity [8]. A number of studies of adipogenesis have been performed in an effort to reduce the burden of obesity [9,10]. However, more extensive investigations of the roles of PTMs, especially acetylation, in adipogenesis are necessary.

Malate dehydrogenase catalyzes the reversal oxidation of malate into oxaloacetate, utilizing the NAD^+/NADH cofactor system. Mammals have two isozymes-MDH1 (encoded cytosolic enzyme) and MDH2 (encoded mitochondrial enzyme) [11]. MDH1 is localized in cytosol and may play a pivotal role in the malate-aspartate shuttle, which in turn influences the metabolic coordinator between cytosol and mitochondria. On the other hand, the mitochondrial enzyme, MDH2, plays a key role in the TCA cycle. Recently, we reported a variety of acetylated proteins during the adipogenic differentiation of 3T3-L1 preadipocyte cells [4]. We also found that the acetylation of MDH1 is increased during adipogenesis and that acetylation enhances its enzymatic activity. Activated MDH1 supports acetyl-CoA and NADPH for fatty acid synthesis [4]. Here, we examine the effects of MDH2 acetylation on adipogenesis.

2. Materials and methods

2.1. Cell culturing and adipogenic differentiation

3T3-L1 cells derived from mouse embryonic fibroblasts were purchased from ATCC. The cells were cultured in a growth medium (high-glucose DMEM containing a 1% antibiotic–antimycotic solution and 10% bovine calf serum; Gibco-Invitrogen, Carlsbad, CA) at 37 °C in a humidified atmosphere with 5% CO_2. GP2-293 packaging cells were grown in DMEM containing a 1% antibiotic–antimycotic solution and 10% FBS. 3T3-L1 cells were induced to differentiate

* Corresponding authors. Fax: +82 42 860 4593 (S.C. Lee). Address: Research Center for Integrated Cellulomics, KRIBB, Daejeon 305-806, South Korea. Fax: +82 42 860 4593 (K.-H. Bae).
E-mail addresses: lesach@kribb.re.kr (S.C. Lee), khbae@kribb.re.kr (K.-H. Bae).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.016

into mature adipocytes, as described in our previous reports [4,10]. Confluent 3T3-L1 cells were incubated in a differentiation medium composed of DMEM, 10% FBS, and MDI (a differentiation cocktail of 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 10 μg/ml insulin [Sigma, US]). After 48 h, the medium was changed to a maintenance medium (DMEM, 10% FBS, and 10 μg/ml insulin). The medium was replenished every other day [10].

2.2. Oil-Red-O staining

Differentiated cells were washed twice with PBS and fixed with 10% formalin for 30 min at room temperature. The cells were then washed with distilled water and stained for 30 min at room temperature with 0.3% filtered Oil-Red-O solution in 60% isopropanol (Sigma, St. Louis, MO). The stained cells were washed with distilled water, and micrographs were obtained. To extract the incorporated Oil-Red-O dye, absolute isopropanol was added to the stained culture dish, and the dish was shaken at room temperature for 30 min. Triplicate samples were read at 510 nm using a GeneQuant 1300 spectrophotometer (GE HealthCare, Uppsala, Sweden) [10,12].

2.3. Construction of retroviral vectors and transduction

To construct 3T3-L1 cells that stably express a FLAG-tagged wild-type or mutant MDH2 protein, a retroviral infection system was used. For the expression of MDH2, DNA encoding the FLAG-tagged MDH2 was inserted into 3T3-L1 cells using the pRetroX-IRES-ZsGreen1 vector (Clontech). For virus production, GP2-293 cell lines were transfected using Lipofectamine 2000 (Gibco-Invitrogen). The details of the transfection and transduction methods are described in our previous reports [4]. Infected cells were selected using a FACSAria cell sorter (BD Biosciences, San Jose, CA) and were further maintained in a growth medium. The ectopic expression of MDH2 was confirmed by a Western blot analysis using anti-FLAG and anti-MDH2 antibodies.

2.4. Introduction of mutations at putative acetylation sites

FLAG-tagged MDH2 was mutated using the EZchange™ Site-Directed Mutagenesis kit (Enzynomics, KR) [13]. The putative acetylation sites in MDH2 were mutated to arginine. Arginine is charged and abolishes acetylation. The sites mutated in this study were based on those reported by Zhao et al. [14] (K301, K307 and K314). We introduced this type of mutation at all three sites of MDH2.

2.5. Activity assay of MDH2

The cells were washed twice with ice-cold PBS and lysed by an extraction buffer (MDH2 activity assay kit; Abcam, Cambridge, UK) containing KDAC inhibitors (10 μM trichostatin A, 10 mM nicotinamide and 50 μM butyric acid; Sigma). After centrifugation, a

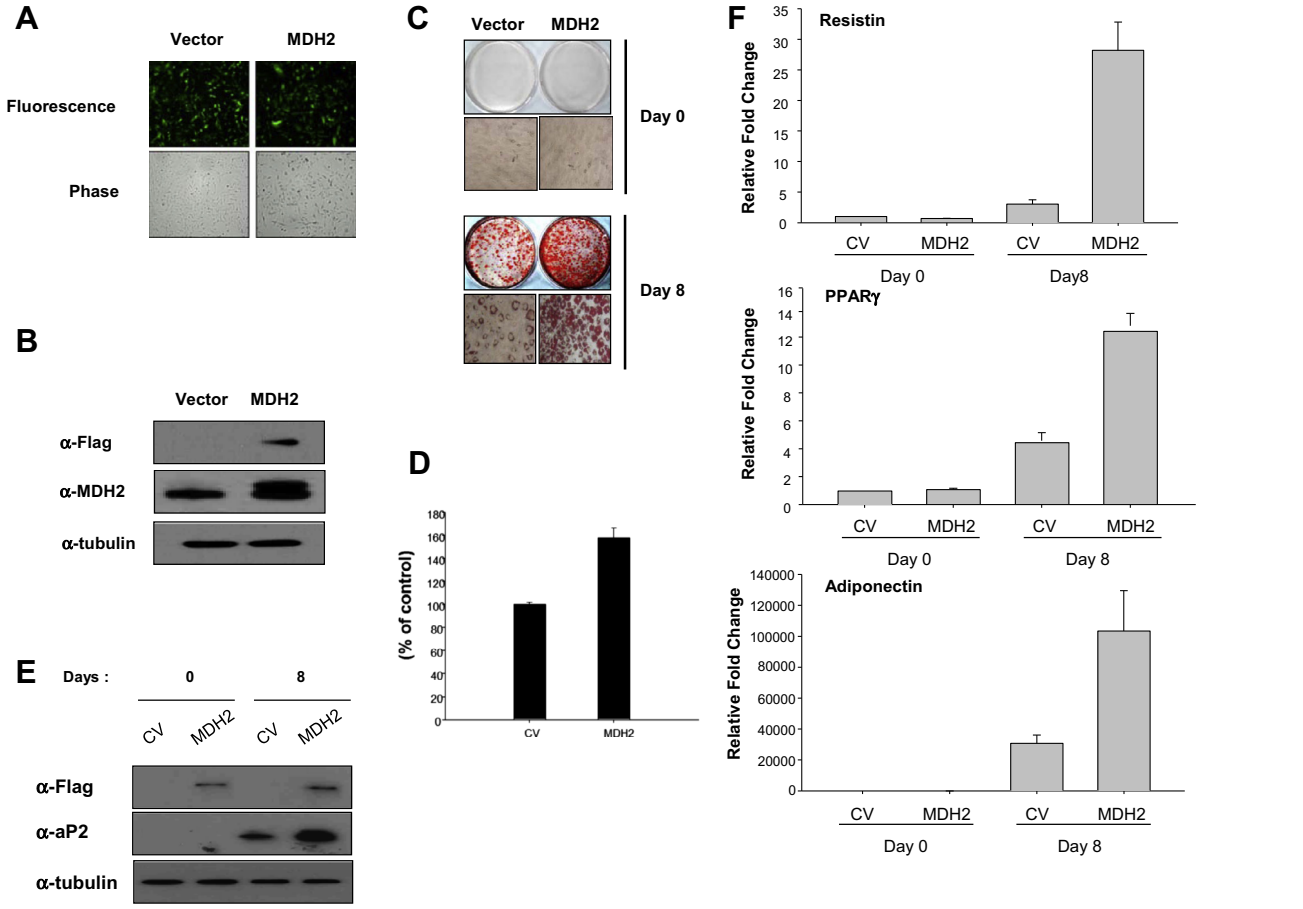

Fig. 1. The ectopic expression of MDH2 accelerates the adipogenic differentiation of 3T3-L1 cells. (A) GFP expression levels of cells were examined under a fluorescence microscope. (B) The ectopic expression of MDH2 was confirmed using an anti-FLAG antibody. (C) The enriched cells were differentiated into adipocytes for 8 days, after which the cells were stained with Oil-Red-O to assess the lipid droplets. (D) Quantification of stained cells was carried out using a dye extraction buffer. (E) The maintenance of the ectopic expression of MDH2 was tested at day 0 and day 8 after adipogenic differentiation. α-Tubulin was used as a loading control. (F) The expression levels of adipogenic markers, specifically resistin, PPARγ and adiponectin, were checked using real-time PCR.

quantitative analysis of the supernatants was performed using a Bradford assay (Bio-Rad). 100 μl of each diluted sample was added into each well and the samples were incubated for 3 h at room temperature. Each well was washed twice with 300 μl of a wash buffer. Next, the MDH2-coated wells were reacted with an activity solution. The reaction was monitored on a microplate reader at 450 nm with shaking.

2.6. Analysis of intracellular NADPH levels

The NADP/NADPH assay kits (Abcam, Cambridge, UK) were used to measure the intracellular NADPH level. The cells were washed three times with PBS and placed in NADP/NADPH extraction buffer. The washed cells were extracted by two freeze (20 min on dry-ice)/thaw (10 min at room temperature) cycles, after which the cell lysates were suspended with buffer using gentle sonication. The concentration of the eluted proteins was checked using a Bradford assay (Bio-Rad). An NADPH standard was made using a serial dilution of NADPH. The NADPH reaction was monitored on a microplate reader for 1–4 h at 450 nm.

3. Results

3.1. The ectopic expression of MDH2 induces the acceleration of adipogenesis

Through an acetylome analysis during the adipogenesis of the 3T3-L1 cells, a variety of proteins showing differential acetylation patterns were obtained. Among these, the acetylation level of MDH2 was dramatically enhanced during adipogenesis, although the expression level also slightly increased [14]. To clarify the functional roles of MDH2 acetylation, we examined the effect of the ectopic expression of MDH2 on adipogenic differentiation as an initial step. 3T3-L1 cells were infected with FLAG-tagged full-length human MDH2 using a retroviral expression system (IRES-GFP), after which only infected cells were enriched by a FACS sorter. As shown in Fig. 1A, most of the cells were GFP-positive, indicating that the MDH2 was well expressed in most cells. The ectopic expression of MDH2 was also revalidated by a Western blot analysis (Fig. 1B). The enriched cells were induced to differentiate into adipocytes by culturing with a differentiation medium. The ectopic expression of MDH2 significantly promotes adipogenic differentiation compared to that with a vector control (Fig. 1C and D). The MDH2 ectopic expression level was continuously maintained until the late stage of adipogenic differentiation (Fig. 1E). The expression level of aP2, a typical adipogenic differentiation marker, was dramatically enhanced at a late stage of differentiation when MDH2 was introduced. In addition, several adipogenic differentiation markers, in this case resistin, PPARγ, and adiponectin, were also increased upon the ectopic expression of MDH2. These results strongly suggest that MDH2 is closely involved in adipogenic differentiation.

3.2. The acetylation level of MDH2 is dependent on the extracellular glucose concentration

Acetyl-CoA is used as source of protein acetylation, and the level of acetyl-CoA is strongly affected by the extracellular glucose concentration. Thus, we checked if the extracellular glucose concentration has an effect on the acetylation level of MDH2 during adipogenesis. As shown in Fig. 2A and B, the higher the extracellular glucose concentration, the better adipogenic differentiation was detected. The acetylation level of MDH2 was also well correlated with the glucose concentration (Fig. 2C and D). Interestingly, the expression level of MDH2 at a low glucose concentration was decreased compared to that of at high glucose concentration. These results clearly suggest that the acetylation of MDH2 is very closely related to the extracellular glucose concentration.

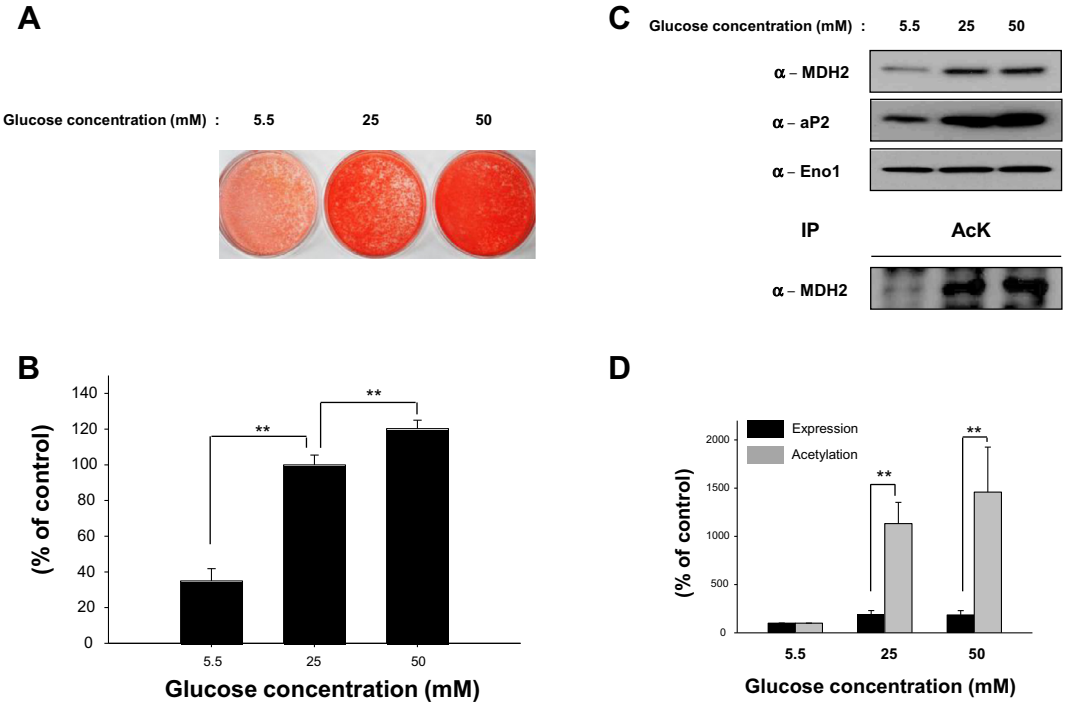

Fig. 2. MDH2 acetylation depends on the extracellular glucose concentration. (A) The 3T3-L1 cells were differentiated into adipocytes at various glucose concentrations. At 12 days of differentiation, the cells were stained with Oil-Red-O. (B) Quantification of stained cells was carried out using a dye extraction buffer. (C) MDH2 acetylation was measured by a Western blot analysis using an anti-acetyllysine antibody. (D) Quantification of the expression and acetylation levels of MDH2 at several glucose concentrations. The quantification value was calculated based on the intensity of (C).

3.3. The enzymatic activity is enhanced by acetylation of MDH2

To clarify the roles of MDH2 acetylation, we constructed acetylation-block mutant MDH2 and then transducted it into 3T3-L1 cells using a retroviral expression system (Fig. 3A). The putative acetylation sites K301, K307 and K314 were chosen for mutation to arginine, with the term MDH2-3KR. The expression of MDH2-3KR was confirmed using a Western blot analysis (Fig. 3B). The transduced cells were differentiated into adipocytes by culturing with a differentiation medium. 3T3-L1 cells expressing MDH2-3KR showed significant reduced adipogenic differentiation compared to that of wild-type MDH2 (Fig. 3C and D), indicating the importance of the acetylation of MDH2. The expression of MDH2-3KR was continuously maintained until a late stage of adipogenic differentiation (Fig. 3E). Notably, the expression level of aP2, a typical adipogenic marker, was also reduced upon MDH2-3KR expression. In addition, the acetylation level of MDH2-3KR showed a dramatic decrease compared to that of wild-type MDH2 (Fig. 3F). The adipogenic differentiation markers, resistin, PPARγ and adiponectin, were also significantly reduced compared to wild-type MDH2. These results revealed that the acetylation of MDH2 is crucial for adipogenic differentiation.

Next, we tested the specific activity of MDH2 to examine if acetylation has an effect on MDH2 enzymatic activity. As shown in Fig. 4A, the ectopically expressed wild-type MDH2 showed higher specific activity than MDH2-3KR at a late stage of adipogenic differentiation. This may have been due to the acetylation of MDH2. Additionally, we examined the intracellular NADPH level. The ectopic expression of wild-type MDH2 induces an increased intracellular NADPH level (Fig. 4B).

4. Discussion

A variety of evidence suggests that metabolic pathways are finely coordinated via the reversible acetylation of metabolic enzymes in response to the state of nutrient availability [14,15]. The reversible acetylation of metabolic enzymes can occur immediately and can thus induce a rapid metabolic switch compared to transcriptional regulation. Protein acetylation is catalyzed by lysine acetyltransferases (KATs) which use acetyl-CoA as an acetylation donor. It has been reported that changes in the availability of acetyl-CoA can directly affect the acetylation status of cellular acetylation substrates [16]. Acetyl-CoA enters the TCA cycle or is used to synthesize fatty acids depending on the energy requirements of the cell. Obesity occurs when energy intake exceeds energy expenditure by hyperplasia (an increased adipocyte number) and hypertrophy (an increase in the size of adipocytes). Therefore, it is reasonable to assume that there are interconnections between adipogenesis and protein lysine acetylation. Recently, we identified the MDH1 as a differentially acetylated protein during the adipogenic differentiation of 3T3-L1 preadipocytes. Additionally, the acetylation of MDH1 increases its enzymatic

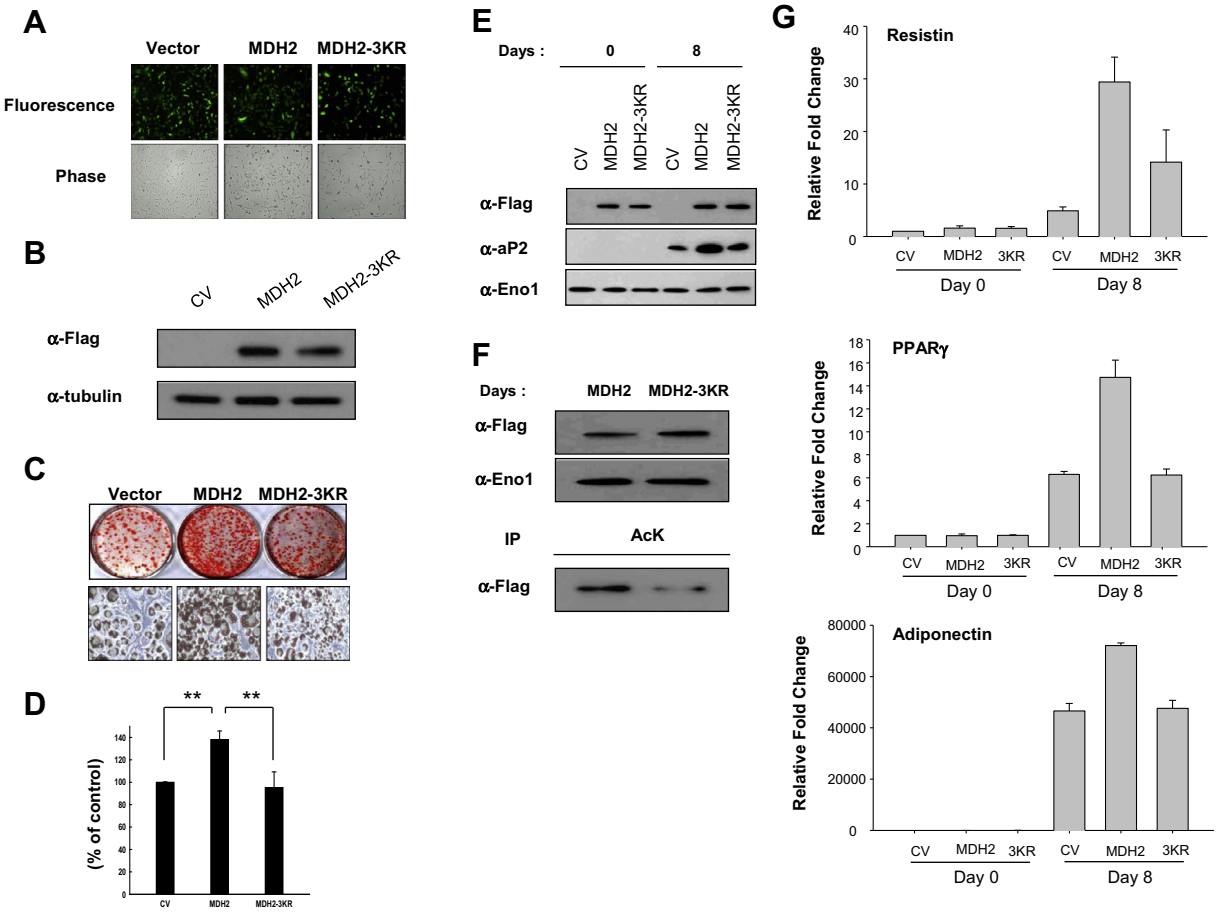

Fig. 3. The mutation effects of the putative acetylation sites of MDH2 on the adipogenic differentiation of 3T3-L1 cells. (A) The GFP expression of cells was examined under a fluorescence microscope. (B) The ectopic expression of the mutant MDH2 was confirmed using an anti-FLAG antibody. (C) The enriched cells were differentiated into adipocytes for 8 days and the cells were then stained with Oil-Red-O. (D) Quantification of stained cells was carried out using a dye extraction buffer. (E) The maintenance of the ectopic expression of MDH2 was tested at day 0 and day 8 after adipogenic differentiation. Enolase was used as a loading control. (F) The MDH2 acetylation level of cells expressing the mutant MDH2 was examined and compared with that of wild-type MDH2. (G) The expression levels of adipogenic the markers resistin, PPARγ and adiponectin were checked using real-time PCR.

activity, and activated MDH1 supports acetyl-CoA and NADPH during fatty acid synthesis by accelerating the citrate shuttle [14]. Here, we investigated the functional roles of MDH2 acetylation on adipogenic differentiation. As shown during the investigation, the acetylation level of MDH2 was dramatically increased. The ectopic expression of MDH2 induced the acceleration of adipogenic differentiation. The mutant MDH2, in which the putative acetylation sites were replaced by arginine, showed significantly suppressed adipogenic differentiation of 3T3-L1 compared to wild-type MDH2, indicating that the acetylation of MDH2 is very important during adipogenic differentiation.

Acetyl-CoA is produced in mitochondria through the β-oxidation of fatty acids and the oxidation of pyruvate to acetyl-CoA [17,18]. When ATP is needed, acetyl-CoA enters the TCA cycle to drive oxidative phosphorylation to replenish ATP. When the ATP supply is sufficient, the acetyl-CoA can be diverted to other purposes, such as energy storage in the form of fatty acids. However, the biosynthesis of fatty acids from acetyl-CoA cannot take place directly because acetyl-CoA is produced in the mitochondria, while fatty acid biosynthesis occurs in the cytosol. There is no mechanism that directly transports acetyl-CoA into the cytosol. To be transported into the cytosol, acetyl-CoA should be converted into citric acid using the pathway of the citrate shuttle system. In the mitochondria, citrate synthase (CS) joins acetyl-CoA with oxaloacetate to make citrate. Then, the citrate is transported from the mitochondria into the cytosol, thus transporting the acetyl-CoA in the form of citrate. In the cytosol, the citrate is converted back into oxaloacetate, which is then reduced to malate by malate dehydrogenase 1 (MDH1). Malate can be oxidized to pyruvate by the malic enzyme, with the production of NADPH, which can contribute to fatty acid biosynthesis [19]. Pyruvate can be re-imported into the mitochondria. Alternatively, malate can be transported itself back into the mitochondria and used to produce NADH once inside the mitochondria.

The reaction of condensation between the methyl carbon of acetyl-CoA and the keto carbon (C-2) of oxaloacetate (OAA) catalyzed by citrate synthase results in the creation of citrate. The standard free energy of the reaction, −8.0 kcal/mol, drives it strongly in the forward direction. Given that the formation of OAA from its precursor, malate, is thermodynamically unfavorable, the highly

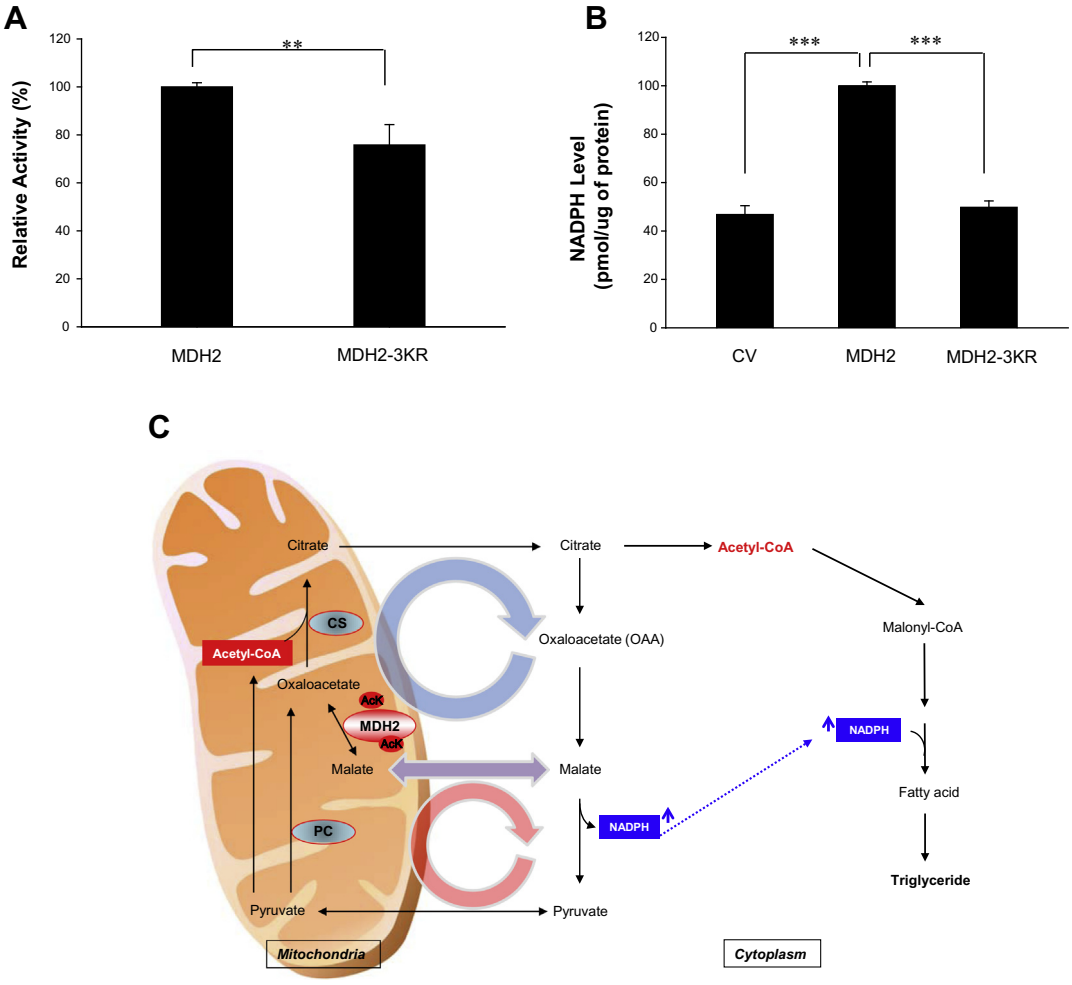

Fig. 4. The MDH2 enzymatic activity and intracellular NADPH level were assessed. (A) The activity levels of the wild-type and MDH2-3KR proteins were assessed after immunopurification using an anti-MDH2 antibody. (B) The intracellular NADPH level was measured using an NADPH assay kit. (C) The proposed mechanism of the roles of MDH2 acetylation during adipogenic differentiation. Through MDH2 acetylation, cytosolic acetyl-CoA and NADPH levels were delicately balanced for efficient fatty acid synthesis. If the mitochondrial OAA level is sufficient and consequently cytosolic acetyl-CoA rises above a certain concentration, acetylated MDH2 efficiently catalyzes the formation of malate from OAA. The malate was translocated into the cytosol, and NADPH was formed by the malic enzyme (red arrow). On the other hand, if the cytosolic acetyl-CoA concentration falls below a certain level, the mitochondrial OAA also has a low concentration. Thus, the acetylated MDH2 catalyzes the formation of OAA from malate, and the formed OAA provides the cytosolic acetyl-CoA via the citrate shuttle (blue arrow). In other words, MDH2 was acetylated when the cellular energy state is sufficient, as in the condition of adipogenic differentiation. The acetylated MDH2 can efficiently balance the levels of cytosolic acetyl-CoA and NADPH for efficient fatty acid synthesis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

exergonic nature of the citrate synthase reaction is of crucial importance in keeping the entire cycle going in the forward direction, as it drives OAA formation by mass action principles. Malate is the specific substrate for MDH2, the final enzyme of the TCA cycle. The forward reaction of the cycle, the oxidation of malate, yields OAA. In the forward direction, the reaction has a standard free energy of about +7 kcal/mol, indicating the very unfavorable nature of the forward direction. As mentioned above, the citrate synthase reaction that condenses oxaloacetate with acetyl-CoA has a standard free energy of about −8 kcal/mol and is responsible for pulling the MDH reaction in the forward direction.

Pyruvate carboxylase (PC) is an enzyme that catalyzes the irreversible carboxylation of pyruvate to form OAA. During adipogenesis, the OAA level is important, as condensation with acetyl-CoA is the only means of transporting acetyl-CoA into the cytosol for the biosynthesis of fatty acids [17]. In mammals, pyruvate carboxylase is expressed in a tissue-specific manner, with its activity found to be highest in the liver, kidney and adipose tissue. It has been reported that the specific activity and expression level of pyruvate carboxylase are dramatically increased during adipogenic differentiation. Through this reaction, sufficient OAA may be prepared to condense with acetyl-CoA to transfer acetyl-CoA into cytosol via the adipogenic differentiation process. Therefore, MDH2 prefers the catalyzation of the reverse reaction, producing malate. Then, malate is translocated into the cytosol and converted into pyruvate by the malic enzyme, leading to the production of NADPH. According to several reports [20,21], MDH2 physically interacts with CS and PC. Collectively, the acetylation of MDH2 during adipogenic differentiation induces an increase in the enzymatic activity and catalyzes the formation of malate more efficiently, resulting in a higher cytosolic NADPH level. These data clearly suggest that the acetylation of MDH2 may be a form of cross-talk between the cellular energy status and fatty acid biosynthesis (Fig. 4C).

In summary, during adipogenesis, the fine regulation of a balance among OAA, acetyl-CoA, malate, NADPH levels in the cytosol and mitochondria may be critical for the efficient biosynthesis of fatty acids. The acetylation of MDH2 can induce enhanced enzymatic activity. Therefore, the regulation of MDH2 activity by acetylation is one of the most important regulation methods for balancing these metabolites during adipogenesis.

Acknowledgments

We thank Drs. Sung Goo Park and Sayeon Cho for their continuous encouragement and helpful advice. In addition, we are grateful to Professor Sang J. Chung for the careful reading of the manuscript and the insightful comments. This work was supported by grants from KRIBB and the research program (Grants 2013M3A9A7046301, 2012M3A9C7050101 and 2011-0030028) of the Korea National Research Foundation.

References

[1] J.W. Locasale, L.C. Cantley, Metabolic flux and the regulation of mammalian cell growth, Cell Metab. 14 (2011) 443–451.
[2] A. Norvell, S.B. McMahon, Cell biology. Rise of the rival, Science 327 (2010) 964–965.
[3] K.-L. Guan, Y. Xiong, Regulation of intermediary metabolism by protein acetylation, Trends Biochem. Sci. 36 (2011) 108–116.
[4] E.Y. Kim, W.K. Kim, H.J. Kang, J.H. Kim, S.J. Chung, et al., Acetylation of malate dehydrogenase 1 promotes adipogenic differentiation via activating its enzymatic activity, J. Lipid Res. 53 (2012) 1864–1876.
[5] B. Schwer, M. Eckersdorff, Y. Li, J.C. Silva, D. Fermin, et al., Calorie restriction alters mitochondrial protein acetylation, Aging Cell 8 (2009) 604–606.
[6] S. Xing, Y. Poirier, The protein acetylome and the regulation of metabolism, Trends Plant Sci. 17 (2012) 423–430.
[7] J.M. Natambi, Y. Kim, Adipocyte differentiation and gene expression, J. Nutr. 130 (2000) 3122–3126.
[8] K.-H. Bae, W.K. Kim, S.C. Lee, Involvement of protein tyrosine phosphatases in adipogenesis: new anti-obesity targets?, BMB Rep 45 (2012) 700–706.
[9] A. Iyer, D.P. Fairlie, L. Brown, Lysine acetylation in obesity, diabetes and metabolic disease, Immunol. Cell Biol. 90 (2012) 39–46.
[10] W.K. Kim, H. Jung, E.Y. Kim, D.H. Kim, Y.S. Cho, et al., RPTP tyrosine phosphatase promotes adipogenic differentiation via modulation of p120 catenin phosphorylation, Mol. Biol. Cell 22 (2011) 4883–4891.
[11] R.A. Musrati, M. Kollárová, D. Mikulásová, Malate dehydrogenase: distribution, function and properties, Gen. Physiol. Biophys. 17 (1998) 193–210.
[12] M.-H. Moon, J.-K. Jeong, J.-H. Lee, Y.-G. Park, Y.-J. Lee, et al., Antiobesity activity of a sphingosine 1-phosphate analogue FTY720 observed in adipocytes and obese mouse model, Exp. Mol. Med. 44 (2012) 603–614.
[13] H.-R. Choi, W.K. Kim, E.Y. Kim, B.S. Han, J.-K. Min, et al., Dual-specificity phosphatase 10 controls brown adipocyte differentiation by modulating the phosphorylation of p38 mitogen-activated protein kinase, PLoS One 8 (2013) e72340.
[14] S. Zhao, W. Xu, W. Jiang, W. Yu, Y. Lin, et al., Regulation of cellular metabolism by protein lysine acetylation, Science 327 (2010) 1000–1004.
[15] C. Choudhary, C. Kumar, F. Gnad, M.L. Nielsen, M. Rehman, et al., Lysine acetylation targets protein complexes and co-regulates major cellular functions, Science 325 (2009) 834–840.
[16] Q. Wang, Y. Zhang, C. Yang, H. Xiong, Y. Lin, et al., Acetylatioin of metabolic enzymes coordinates carbon source utilization and metabolic flux, Science 327 (2010) 1004–1007.
[17] D.A. Fell, J.R. Small, Fat synthesis in adipose tissue, Biochem. J. 238 (1986) 781–786.
[18] J.K. Suagee, B.A. Corl, M.V. Crisman, J.G. Wearn, L.J. McCutcheon, et al., *De novo* fatty acid synthesis and NADPH generation in equine adipose and liver tissue, Comp. Biochem. Physiol. B 155 (2010) 322–326.
[19] P. Jiang, W. Du, A. Mancuso, K.E. Wellen, X. Yang, Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence, Nature 493 (2013) 689–693.
[20] S. Beeckmans, L. Kanarek, Demonstration of physical interactions between consecutive enzymes of the citric acid cycle and of the aspartate–malate shuttle, Eur. J. Biochem. 117 (1981) 527–535.
[21] Y. Ho, A. Gruhler, A. Heilbut, G.D. Bader, L. Moore, et al., Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry, Nature 415 (2002) 180–183.